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# CANAVANINE IN *CANAVALIA PARAGUAYENSIS*, *C. GLADIATA* AND *DIOCLEA PARAGUAYENSIS*

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**Key Word Index**—*Canavalia*; *Dioclea*; Leguminosae; canavanine; amino acid; method of isolation.

**Abstract**—A new method for the isolation of canavanine has been developed which has the advantage of handling small samples as well as enabling large scale isolation. *Canavalia paraguayensis*, *C. gladiata* and *Dioclea paraguayensis* are new sources of canavanine.

## INTRODUCTION

CANAVANINE was discovered in 1930 by Kitagawa and Tomiyama[1] in seeds of the jack bean, *Canavalia ensiformis*. The isolation was effected by treatment of the extract with flavianic acid and transformation of the diflavianate via the dipicrate to the free amino acid in about 2·8% yield. Specific colour reactions with ninhydrin, Sakaguchi reagent and pentacyanoammonioferrate reagent[2] provided the means for surveying the distribution of canavanine in the Leguminosae, subfamily Lotoideae.[3–5]

## RESULTS AND DISCUSSION

Paper chromatographic analysis of canavanine in the aqueous methanolic extracts of seeds of *C. paraguayensis*, *C. gladiata* and *Dioclea paraguayensis* showed a similar pattern as *C. ensiformis*. They contained canavanine as predominant amino acid. Its identification was afforded by a new method which we wish to describe because of its simplicity.

Ground seeds were extracted with boiling 70% methanol. The solvent was removed *in vacuo*, the residue diluted with water and the filtrate passed through a column of strong basic anion exchange resin. Elution with acetic acid yielded canavanine acetate which by ion exchange was transformed into the sulfate. It proved to be identical with an authentic sample of canavanine sulfate by IR, m.m.p., $R_f$ and optical rotation.

## EXPERIMENTAL

*Plant material.* Seeds of the above named species were collected by E.B. in the outskirts of Asunción, Paraguay. Herbarium material of the plants has been deposited at Instituto de Botanica Darwinion, San Isidro, Argentina.

*Apparatus.* IR: Leitz-Unicam SP 200 G; m.p.: Kofler (uncorrected); opt. rotation: Zeiss LEP A2 0·005° (photoelectrically in a 1-dm tube).

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*Chromatography.* 10 $\mu$l of 1% methanolic solution of samples of canavanine sulfate were chromatographed on Schleicher–Schüll paper 2043 b in *n*-BuOH–HOAc–$H_2O$ (4:1:5): $R_f$ 0·08; spray reagents: ninhydrin, Sakaguchi, pentacyanoammonioferrate;[2] colours: blue, violet and red-violet respectively.

*Isolation.* 30 g seed portions were crushed and ground in an electric mill to a fine powder. The powder was extracted with three 300-ml portions of boiling 70% MeOH. The combined filtrates were concentrated *in vacuo* to about 50 ml and made up with water to 500 ml. Filtration through a layer of HYFLO Supercel resulted in a clear yellow solution which was run through a column with Dowex (50 ml resin on the $OH^-$ form). The column was washed with 100 ml $H_2O$ and then the absorbed amino acid was eluted with 10% HOAc. 50 ml fractions were collected and checked by PC with pentacyanoammonioferrate reagent. The positive fractions were evaporated *in vacuo* to dryness. The viscous residue was dissolved in 150 ml $H_2O$ and this solution was slowly passed through a column with Dowex 1X2 in the $SO_4^{2-}$ form (40 ml resin). The effluent gave on evaporation a partly crystalline residue. Analytical specimens of canavanine sulfate were produced by two additional recrystallizations from aq. MeOH. Drying under vacuum gave yields between 2·2 and 2·9%. M.p. 172–174°, m.m.p. 172–174°, $C_5H_{12}N_4O_3 \cdot H_2SO_4$. Required: C, 21·89; H, 5·15; N, 20·43 S, 11·69. Found: C, 21·61; H, 5·00; N, 20·38; S, 11·37%; $[\alpha]_{20}^{D}$ +19·8° (*c*, 1·2; $H_2O$); The IR spectrum (i; KBr) was coincident with the spectrum of an authentic sample.